Tailoring drug release profile of low-molecular-weight hydrogels by supramolecular co-assembly and thiol–ene orthogonal coupling†

David Díaz Díaz,*[ab] Emmanuelle Morin,[cd] Eva M. Schön,[a] Ghyslain Budin,[d] Alain Wagner[d] and Jean-Serge Remy[c]

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We describe a general user-friendly platform for fine-tuning the drug release properties of low-molecular-weight hydrogels by a combination of supramolecular co-assembly of complementary molecular structures and controlled photochemical thiol–ene cross-linking. Other critical features such as thermomechanical stability and morphology of the nanostructured hydrogels are also tailored by this approach.

Molecular functional gels able to immobilize a large number of solvent molecules have been a subject of study for well over a century.[1] These hierarchical, self-assembled, and viscoelastic materials may be considered to be either hard or soft based on their rheological characteristics,[2] and can be categorized into two major types according to their driving forces for molecular aggregation: chemical gels,[3] based on covalent bonds, and physical gels,[1,4] based on non-covalent bonds. 'Bottom-up' processing of stable and stimuli-responsive gels has allowed their use in important applications (*e.g.* regenerative medicine, sensors, nanoelectronics, *etc.*).[5] Nevertheless, the search for a universal platform to fine-tune their functional properties, especially in the case of gels made of low-molecular-weight-gelators (LMWGs), continues being a great scientific challenge for the creation of shape-controlled and robust functional soft-materials.[6–8] On the other hand, Sharpless and co-workers introduced, early in this decade, the valuable concept of 'click' chemistry,[9] exemplified by the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[10] After the first 'boom' caused by the versatility of the CuAAC,[11] the century-old thiol–ene coupling (TEC)[12] has emerged as a competitive orthogonal strategy for the high-yield synthesis of complex functional networks under mild conditions.[13,14]

In this communication, we report the supramolecular co-assembly (SMCA) of complementary structures followed by controlled TEC as a new user-friendly strategy for fine-tuning the drug release kinetics of self-assembled hydrogels made of LMWGs. In addition, its effect on the *sol*-to-*gel* transition temperature (T_{gel}) and morphology of the materials is also described.

The hydrogelation ability of LMW hydrogelator dibenzoyl-L-cystine (**1**) at very low concentrations (0.2 wt%) was already noted in 1892[15] and revisited by Menger and Caran in the late 90's.[16] The gelation phenomenon is driven here by a favorable backbone orientation (CH_2–S–S–CH_2 dihedral angle ~87 to 99°) enhanced by cooperative hydrogen-bonding and π–π stacking interactions (Fig. 1). Inspired by our previous studies directed towards the mechanical stabilization of organogels *via* CuAAC,[17] pseudo-complementary compounds **2–4** were synthesized by incorporation of terminal alkene units.[18] In contrast to other LMWGs,[19] the simple co-assembly process of LMWG **1** at 0.2 wt% in water with the corresponding alkene-containing analogues **2**, **3** or **4** (under optimized molar ratio **1** : (**2–4**) = 10 : 1) did not improve the mechanical strength of the fragile original hydrogel.[18]

The stability of the hydrogels was not enhanced either when they were made of the mixtures **1** : (**2–4**) in the presence of polyvalent thiol-containing cross-linkers **5–7** (under molar ratio alkene groups : thiol groups = 1 : 1) and a water-soluble photoinitiator (*i.e.* lithium phenyl-2,4,6-trimethylbenzoyl phosphinate (LAP), 0.05 wt% respect to the thiol monomer).[18] Such fragile gel-like materials were easily disrupted either upon hand shaking after cooling down the isotropic solutions or after static standing for one week at 293 K. Nevertheless, UV-light irradiation (λ = 365 nm) of the above mixtures during 10 min induced the TEC cross-linking and hence the

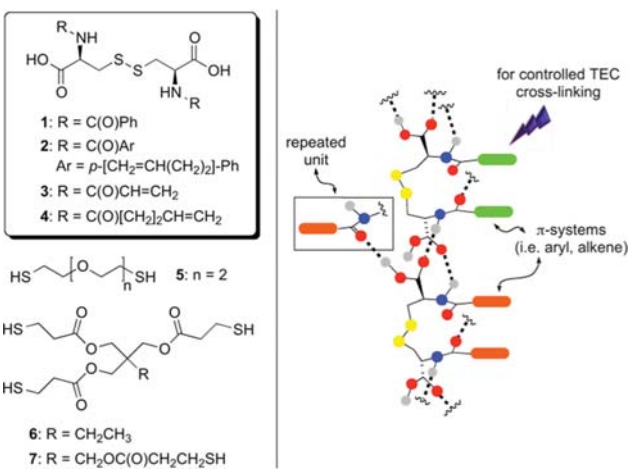

Fig. 1 *Left*: chemical structures of L-cystine-based LMWG **1**, alkene-containing analogues **2–4**, and polyvalent thiol cross-linkers **5–7**. *Right*: plausible self-assembly pattern of mixtures **1–4** based on previous X-ray crystal structure[15] (spheres: red = oxygen; blue = nitrogen; yellow = sulfur; grey = hydrogen; dotted lines = hydrogen bonds between amide-NH and carboxyl-CO groups).

[a]*Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, 93040 Regensburg, Germany. E-mail: David.Diaz@chemie.uni-regensburg.de; Fax: +49 941 9434121; Tel: +49 941 9434373*
[b]*Instituto de Ciencias de Materiales de Aragón, CSIC-Universidad de Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain*
[c]*Chimie Génétique, Faculté de Pharmacie, 67000 Strasbourg, France*
[d]*Laboratory of Functionnal ChemoSystems, UMR 7199, Faculté de Pharmacie, 67000 Strasbourg, France*

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development of robuster viscoelastic gel phases, as demonstrated by both oscillatory rheological and thermodynamic measurements (Table 1). Under the above experimental conditions neither hydrolysis nor reductive degradation of the activated amidic groups were appreciable. A molar ratio **1** : (**2–4**) = 10 : 1 was found to be optimum since the temporal stability of the gels was found to decrease significantly at higher concentrations of **2–4**. Besides the additional absorption bands characteristic of the additives used in the modification of the gels, no major information could unequivocally be acquired from standard infrared studies.

Appropriate control experiments demonstrated the cooperative effect of SMCA and TEC on the gel stability (Fig. 2).[18] The modified thermoreversible hydrogels could be sterilized by a trituration/washing cyclical protocol and final restoration in milli-Q-purified water. The thermodynamic properties of the gels (defined by the absence of flow upon inversion of the test tube) were examined by the estimation of their T_{gel}.[20] Their mechanical strengths were quantified rheologically under oscillating stress. The results confirmed the gel state ($G' > G''$) and britte nature of each sample. In general, the average storage moduli (G') were uniformly found to be about four-fold greater than respective loss moduli (G''). The structure of both compatible analogues **2–4** and cross-linkers **5–7** was found to play a key role on the physical properties of the final materials. Thus, the higher enhancement of the T_{gel} (up to 33 °C respect to the unmodified gel, Table 1, entry 1) was found by using **2** and **6** as additives (Table 1, entry 3). Among the LMWG analogues **2–4**, compound **2** was found to be the most effective in stabilizing the gel network without losing the thermoreversible function (Table 1, entries 2 and 3), presumably due to the preservation of the aromatic ring in its structure, which helps for a better intercalation of **2** along the gel fibers made of **1**. In contrast, acryloyl derivative **3**, lacking the aromatic ring, provided inconsistent results resulting from its higher tendency to polymerize. This could be controlled by breaking the conjugation of the vinyl group as in compound **4**, which afforded a modest increment of the T_{gel} of *ca.* 20 °C upon SMCA–TEC (Table 1, entry 4). Among different cross-linkers, trivalent thiol **6** was found to be the most effective followed by PEG-bifunctional thiols (*e.g.* **5**).[18] The use of tetravalent thiol **7** leads to unhomogenous samples due to partial crystallization upon TEC. The actual effect of the TEC on the gel strength was confirmed by rheological measurements of the materials made of **1** + **2** + **5**, **1** + **2** + **6** or **1** + **4** + **6** before irradiation, which did not show any improvement of the storage moduli compared to the original gel.[18]

The microstructure of the modified hydrogels was investigated by transmission and scanning electron microscopy (TEM and SEM respectively). As previously observed,[16] gels made of LMWG **1** self-assemble into μm length fibers of 30–150 nm in diameter (Fig. 3A and B). Gels modified by the SMCA–TEC approach showed a μm scale porous network of varying morphologies, including large areas covered by porous clusters and wrinkled laminated structures that may account for the opacity of the modified gels (Fig. 3C–I). Depending on the composition mixture used during SMCA–TEC, certain degree of conservation during the transcription of the original supramolecular structure could be achieved (Fig. 3D and G), suggesting that their observed greater stabilities were not the result, at least exclusively, of very large changes in the microstructure.

It is important to remark that in comparison with other 'click'-type reactions, such as CuAAC,[17] orthogonal TEC may offer a much

Table 1 Representative thermodynamic and rheological data of LMWG **1**-based original hydrogel and modified hydrogels *via* SMCA–TEC[a]

Entry	Components	T_{gel}[c]	G'[d]/Pa	G''[e]/Pa	γ_c[f] (%)
1	**1**	29	57.8	15.7	0.407
2	**1** + **2** + **5**[b]	55	77.5	18.7	0.675
3	**1** + **2** + **6**[b]	62	89.6	20.7	0.792
4	**1** + **4** + **6**[b]	48	71.3	17.1	0.579

[a] Concentration of **1** = 0.2 wt%. Molar ratios of components as indicated in text. The use of 0.5 wt% of DMSO in the solvent system facilitated the homogenization of the mixture prior TEC. Modified gels were purified prior characterization. Reaction conversion was confirmed by the lack of double bond signals in crude NMR analyses. [b] LAP was used as water-soluble photoinitiator. [c] *Sol*-to-*gel* transition temperature (±3 °C). [d] G' = average storage modulus (±1.2 Pa). [e] G'' = average loss modulus (±0.05 Pa). [f] Critical strain amplitude, above which the gels fracture (±0.01).

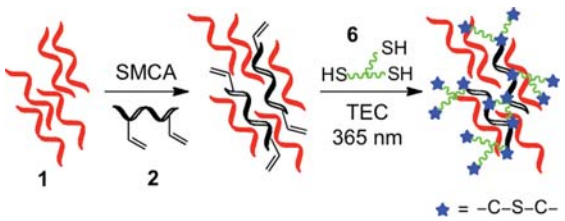

Fig. 2 General SMCA–TEC strategy for fine-tuning the properties of LMW hydrogels. Model components: **1** = LMW hydrogelator **1**; **2** = alkene-containing complementary compound **2**; **6** = trivalent thiol cross-linker **6**. A detailed description of the chemistry involved in both processes can be found in the ESI†.

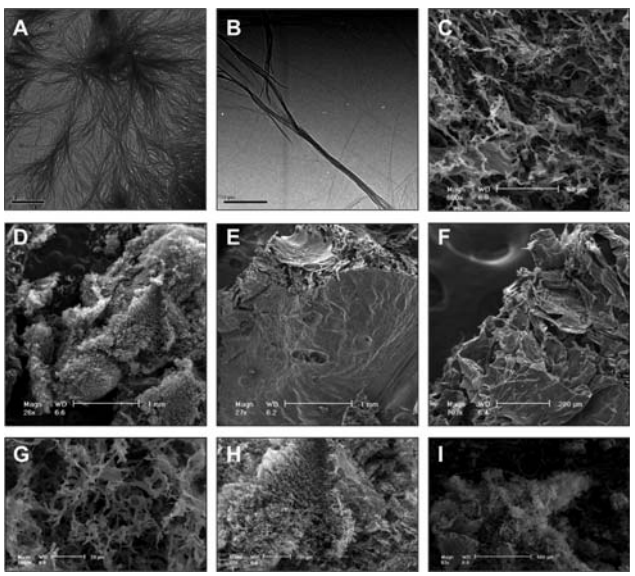

Fig. 3 Representative electron microscopy photographs of gels outlined in Table 1: (A and B) TEM images of fibrilar network and individual entwined fibers of the hydrogel made of **1** (bars: A = 2 μm, B = 1 μm); (C, D, G, H, and I) SEM images of the cross-linked xerogel from **1** + **2** + **6** (bars: C = 50 μm, D = 1 mm, G = 20 μm, H = 200 μm, I = 500 μm); (E and F) SEM images of the cross-linked xerogel from **1** + **2** + **5** (bars: E = 1 mm, F = 200 μm).

faster and controllable cross-linking process in the absence of metal-catalysts and under aerobic conditions, which markedly facilitates practical syntheses of gel-based materials. In addition, another attractive feature of the SMCA–TEC approach is that a number of important functions of the gels are, in principle, susceptible to be tailored by controlling the reaction parameters (*i.e.* additives structure, molar concentrations, irradiation conditions). As a proof of concept, and as part of our research program focused on engineering tunable materials for biomedical applications, we investigated the influence of the above parameters on the release properties of small drugs from the modified hydrogels.[21] For convenience, drug entrapment and release experiments were carried out *in vitro* using 2-hydroxyquinoline as a model water-soluble and UV-active drug, which is structurally unrelated to the gel matrix.[22] 2-Hydroxyquinoline (1 mM) was physically entrapped within the corresponding gel phase as a result of the *sol*-to-*gel* process, and static release studies over time were performed by UV-vis spectroscopic analysis of a liquid phase in contact with the preformed gels as indicated in Table 1. Although it is known that T_{gel} can be increased to some extent in function of the amount of entrapped drug,[22] we did not observed any noticeable effect on the gel properties (*i.e.* visual aspect, T_{gel}, gel strength, kinetics of gel formation) when the drug concentration was adjusted to 1 mM, with only minor variations within the experimental error of the thermodynamic and rheological measurements. Therefore, it seems that any effect of the encapsulated drug on the properties of the hydrogels under the reported conditions is rather small and may be attributed to non-specific interactions between the drug and the LMWG, causing minor changes in the 3D network structure of the doped hydrogels.

The release percentages of the studied gels showed that the drug concentration in the liquid phase reached a plateau value (*ca.* 20–30% of the original embedded drug) within the range of 6–9 h, corresponding to the equilibrium state between drug molecules in solution and entrapped in the gel phase under non-sink conditions.[18] Fig. 4 (top) shows the release and kinetics plots for the drug delivery from the different hydrogels. In each case, the drug release monitored during 14 h was faster than the degradation of the gel. Over this period of time, neither shrinkage nor swelling of the hydrogels were visually perceptible. Despite the slow and continuous increase of the released amount for all gels, the final equilibrium value in static conditions could not be accurately determined by the Higuchi equations[23] due to visible degradation of these gels after 14 h.[18] Both van der Waals and hydrogen bonding interactions between the drug and the 3D gel network establish the release profile for each case when the process is not triggered by increasing the temperature above T_{gel} or basifying the medium.[18] An acceptable correlation between the drug release rate and the expected cross-link density of the modified hydrogels was found before their appreciable degradation. In general, lower release rates were consistently observed for denser materials (Fig. 4 top, entries 2–4 *vs.* 1), which can be explained by the most obstructed pathways that the drug must overcome. Furthermore, the linear relationships ($r^2 > 0.98$) between the released amount of drug and the square root of time (Fig. 4 bottom) support a Fickian diffusion mechanism through the porous structure of the hydrogel carriers.[23] In addition, an inverse linear relationship between release rate and thermo-mechanical stability of the material was generally observed (Fig. 4, entries 3–2 *vs.* 1). Nevertheless, such correlation was found to be meaningless when comparing systems made from different SMCA and TEC processes (Fig. 4, entry 4 *vs.* 2). This could

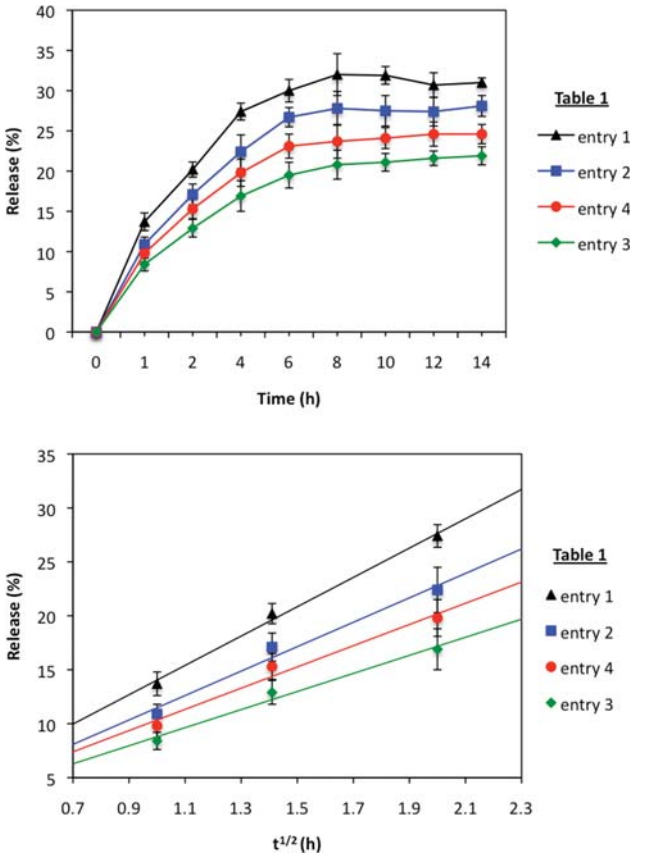

Fig. 4 Release (*top*) and kinetics (*bottom*) profiles of 2-hydroxyquinoline over time at RT from hydrogels prepared as outlined in Table 1. Thus, the composition of the material for each curve is as following: entry 1 = hydrogel made of **1**; entry 2 = cross-linked hydrogel made of **1** + **2** + **5**; entry 3 = cross-linked hydrogel made of **1** + **2** + **6**, and entry 4 = cross-linked hydrogel made of **1** + **4** + **6**. Water was used as receiving medium. Drug concentration in the gels before release = 1 mM.

be explained by the formation of transient networks with junction areas of higher flexibility in the case of divalent cross-linkers.

Drawing correlations between the release rates and the morphology of the gels investigated by SEM can be very risky because the samples have been stressed by freezing-drying, which may lead to freeze concentration effects and thus may not be representative of the real hydrated sample. On the other hand, it is well documented that the release rate of a molecule through a hydrogel matrix can be correlated not only with the storage modulus of the gel but also with the mesh size of the network, surface area to volume ratios, loading and solubility of the studied molecule.[18,24,25] In gels made of LMWGs, the mesh size depends, among other factors, on the history of the self-assembly process, nature and concentration of gelator and/or additives, pH, *etc.*[18,24] Hence, we calculated the diffusion coefficients (D) ($\times 10^{-9}$ m^2 s^{-1}) of the studied materials obtaining the following values: $D_1 = 2.98$, $D_2 = 2.21$, $D_3 = 1.44$, and $D_4 = 1.97$ (subscripts 1–4 indicate the entry in Table 1).[18] These values are comparable to those of small model drugs in other LMW gelling systems.[24,26] The above variation in the diffusion coefficients denotes that 2-hydroxyquinoline interacts differently with each gel matrix upon SMCA–TEC and agrees with the observed differences in rheological behaviour. In agreement with previous works,[27] the higher the interaction between drug and matrix, the lower the

diffusion coefficient and the release rate (Fig. 4), suggesting that the drug is retained by some gels more than others.

In summary, we have described a robust and friendly user platform for fine-tuning the drug release profiles of bioactive substances from LMW hydrogels by a modular approach involving (1) SMCA of LMWGs with complementary molecular units bearing terminal alkenes and (2) subsequent controlled photochemical TEC cross-linking with polyvalent thiol compounds. The observed variation in the diffusion coefficients of a model drug in the different gel matrices upon SMCA–TEC agrees with both differences in rheological behaviour of the material and the release rate of the drug. Moreover, both T_{gel} and nanostructures of the hydrogels prepared at their minimum gelation concentrations can be also modulated by this approach. In principle, other important properties such as oxygen transport ability of gel-based soft films should be susceptible to modification by SMCA–TEC. The attachment of fluorescent dyes to these materials and their study by confocal microscopy are now planned within our research activities. In addition, the assessment of this strategy under physiological conditions and to other systems such as organic and polymer gels designed for specific biomedical applications is currently ongoing in our laboratories.

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